

IT IS CLAIMED:

1. A method of prolonging the survival time of human stem cells in culture, comprising:

- 5       (a) obtaining a population of cells containing human stem cells from a subject;  
         (b) enriching for the human stem cells in said population; and  
         (c) exposing the enriched stem cell population, *ex vivo*, to an oligomer antisense to TGF- $\beta$ , under culture conditions and for a period of time effective to block the effect of TGF- $\beta$  on replication and/or differentiation of said stem cells, wherein the viability and  
10      differentiation state of the stem cells is preserved longer than stem cells not subjected to TGF- $\beta$  antisense treatment.

2. The method of claim 1, wherein the antisense oligomer is a morpholino oligomer characterized by,

- 15     (a) a backbone which is substantially uncharged;  
         (b) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a  $T_m$  greater than 50°C;  
         (c) nuclease resistance; and  
         (d) the capability for active or facilitated transport into cells.

20     3. The method of claim 2, wherein the morpholino antisense oligomer has the phosphorodiamidate linkage represented at Figure 2B, where X=NH<sub>2</sub>, Y=O, and Z=O.

25     4. The method of claim 2, wherein the antisense oligomer has a length of from 12 to 25 bases.

5. The method of claim 3, where the antisense oligomer has a sequence presented as SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:5.

30     6. The method of claim 1, wherein the human stem cells in said enriched stem cell composition are characterized as lacking the expression of lineage markers (*lin-*), and either (a) positive for cell surface expression of CD 34 and KDR and negative for cell surface expression of CD38 or (b) positive for cell surface expression of both CD 34 and Thy1.

7. The method of claim 1, wherein culture conditions effective to preserve the viability and differentiation state of said stem cells means culture in medium lacking endogenously provided cytokines.

5        8. A human stem cell composition capable of rapid *in vivo* reconstitution of the hematopoietic system of a subject comprising,  
a cell population enriched for human stem cells, said stem cells treated *ex vivo* with an oligomer antisense to TGF- $\beta$ , wherein the viability and differentiation state of the stem cells is preserved in culture longer than stem cells not subjected to TGF- $\beta$  antisense treatment.

10      9. The composition of claim 8, wherein the antisense oligomer is a morpholino oligomer characterized by,

- (a) a backbone which is substantially uncharged;
- (b) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C;
- (c) nuclease resistance; and
- (d) the capability for active or facilitated transport into cells.

15      10. The composition of claim 9, wherein the morpholino antisense oligomer has the phosphorodiamidate linkage represented at Figure 2B, where X=NH<sub>2</sub>, Y=O, and Z=O.

20      11. The composition of claim 9, wherein the antisense oligomer has a length of from 12 to 25 bases.

25      12. The composition of claim 10, where the antisense oligomer has a sequence presented as SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:5.

30      13. The composition of claim 8, wherein the human stem cells in said enriched stem cell composition are characterized as lacking the expression of lineage markers (lin-), and either (a) positive for cell surface expression of CD 34 and KDR and negative for cell surface expression of CD38 or (b) positive for cell surface expression of both CD 34 and Thy1.

14. The composition of claim 8, wherein culture conditions effective to preserve the viability and differentiation state of said stem cells means culture in medium lacking endogenously provided cytokines.

5        15. A method of decreasing the time for hematopoietic reconstitution of a patient following chemotherapy or radiation therapy, comprising:

- (a) obtaining a population of cells containing human stem cells from a subject;
- (b) enriching for the human stem cells in said population;
- (c) exposing the enriched stem cell population, *ex vivo*, to an oligomer antisense to TGF- $\beta$ , under culture conditions and for a period of time effective to block the effect of TGF- $\beta$  on replication and/or differentiation of said stem cells;
- (d) culturing the antisense oligomer treated stem cells, wherein the viability and differentiation state of said stem cells is maintained for at least 5 days; and
- (e) administering said cultured TGF- $\beta$  blocking agent-treated stem cells to a subject, wherein the time required for *in vivo* reconstitution of at least one hematopoietic lineage is reduced relative to that of a subject who received stem cells not treated with an oligomer antisense to TGF- $\beta$ .

15        16. The method of claim 15, wherein the human stem cells in said enriched stem cell composition are characterized as lacking the expression of lineage markers (*lin*), and are either (a) positive for cell surface expression of CD 34 and KDR and negative for cell surface expression of CD38 or (b) positive for cell surface expression of both CD 34 and Thy1.

20        17. The method of claim 15, wherein the antisense oligomer is a morpholino oligomer characterized by,

- (a) a backbone which is substantially uncharged;
- (b) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C;
- (c) nuclease resistance; and
- (d) the capability for active or facilitated transport into cells.

25        18. The method of claim 17, wherein the linkage is the phosphorodiamidate linkage represented at Figure 2B, where X=NH<sub>2</sub>, Y=O, and Z=O.

19. A method of rapid *in vitro* production of lineage committed progenitor cells and their progeny from human stem cells, comprising:

- (a) obtaining a population of cells containing human stem cells from a subject;
- (b) enriching for the human stem cells in said population;
- 5 (c) exposing the enriched stem cell population, *in vitro*, to an oligomer antisense to TGF- $\beta$ , under culture conditions and for a period of time effective to block the effect of TGF- $\beta$  on replication and/or differentiation of said stem cells;
- (d) culturing the antisense oligomer treated stem cells, wherein the viability and differentiation state of said stem cells is maintained for at least 5 days; and
- 10 (e) transferring the TGF- $\beta$  antisense-treated stem cells to culture medium containing at least two exogenously provided cytokines or growth factors selected from the group consisting of IL-3, IL-6, IL-11, IL-12, stem cell factor (SCF), flt-3, thrombopoietin (Tpo) and transforming growth factor-beta (TGF-beta), resulting in more rapid production of lineage committed progenitor cells and their progeny than obtained in the absence of TGF- $\beta$  antisense treatment.

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20 20. The method of claim 17, wherein the antisense oligomer is a morpholino oligomer containing,

- (a) a backbone which is substantially uncharged;
- (b) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C;
- (c) nuclease resistance; and
- (d) the capability for active or facilitated transport into cells.

25 21. The method of claim 19, wherein the linkage is the phosphorodiamidate linkage represented at Figure 2B, where X=NH<sub>2</sub>, Y=O, and Z=O.

P.D.W. (A)